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# Virological Outcomes of Second-line Protease Inhibitor–Based Treatment for Human Immunodeficiency Virus Type 1 in a High-Prevalence Rural South African Setting: A Competing-Risks Prospective Cohort Analysis

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**Background.** Second-line antiretroviral therapy (ART) based on ritonavir-boosted protease inhibitors (bPIs) represents the only available option after first-line failure for the majority of individuals living with human immunodeficiency virus (HIV) worldwide. Maximizing their effectiveness is imperative.

**Methods.** This cohort study was nested within the French National Agency for AIDS and Viral Hepatitis Research (ANRS) 12249 Treatment as Prevention (TasP) cluster-randomized trial in rural KwaZulu-Natal, South Africa. We prospectively investigated risk factors for virological failure (VF) of bPI-based ART in the combined study arms. VF was defined by a plasma viral load >1000 copies/mL  $\geq 6$  months after initiating bPI-based ART. Cumulative incidence of VF was estimated and competing risk regression was used to derive the subdistribution hazard ratio (SHR) of the associations between VF and patient clinical and demographic factors, taking into account death and loss to follow-up.

**Results.** One hundred one participants contributed 178.7 person-years of follow-up. Sixty-five percent were female; the median age was 37.4 years. Second-line ART regimens were based on ritonavir-boosted lopinavir, combined with zidovudine or tenofovir plus lamivudine or emtricitabine. The incidence of VF on second-line ART was 12.9 per 100 person-years ( $n = 23$ ), and prevalence of VF at censoring was 17.8%. Thirteen of these 23 (56.5%) virologic failures resuppressed after a median of 8.0 months (interquartile range, 2.8–16.8 months) in this setting where viral load monitoring was available. Tuberculosis treatment was associated with VF (SHR, 11.50 [95% confidence interval, 3.92–33.74];  $P < .001$ ).

**Conclusions.** Second-line VF was frequent in this setting. Resuppression occurred in more than half of failures, highlighting the value of viral load monitoring of second-line ART. Tuberculosis was associated with VF; therefore, novel approaches to optimize the effectiveness of PI-based ART in high-tuberculosis-burden settings are needed.

**Clinical Trials Registration.** NCT01509508.

**Keywords.** HIV; antiretroviral therapy; virological failure; second line; protease inhibitor.

Despite clinical and public health gains following antiretroviral therapy (ART) rollout, treatment failure of first-line nonnucleoside reverse transcriptase inhibitor (NNRTI)–based ART is common [1–4], with up to 3 million human

immunodeficiency virus (HIV)–infected patients estimated to receive second-line, boosted protease inhibitor (bPI)–based ART by 2020 [5]. Treatment failure on second-line ART is a major concern given poor, if any, access to further regimens in high-burden settings.

Data from observational studies of second-line bPI-based ART treatment outcomes in sub-Saharan Africa suggest a 14%–32% prevalence of virological failure (VF) [6–12], with randomized trials from comparable settings reporting lower prevalence of VF at 17%–19% at 48 weeks and longer durations of follow-up [13–16]. These studies were largely conducted in urban or periurban areas. Although associations have been reported between second-line VF and poor adherence [6, 8, 10, 17, 18] and delayed switch [7, 9], socioeconomic and

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demographic factors such as education level, employment, and data on household members have not been studied together. Furthermore, the potentially critical association between concomitant tuberculosis (TB) [17] and second-line bPI failure is inconclusive.

Previously published cohort studies have used a Kaplan-Meier survival analysis and standard Cox regression models that can result in inflated estimates due to competing events. Competing risk regression analysis overcomes this limitation by accounting for events, such as death, that preclude the subject from experiencing the study outcome. This is particularly important in sub-Saharan Africa where mortality remains significant following ART initiation due to advanced disease stage as well as loss to follow-up [19]. Here we have applied competing risk methods to comprehensively and prospectively investigate risk factors for VF of bPI-based second-line ART in a rural population of South Africa. In this setting ART was readily available and follow-up optimum according to local standards, including real-time viral load monitoring.

## MATERIALS AND METHODS

### Study Setting and Study Design

This cohort study was nested within the French National Agency for AIDS and Viral Hepatitis Research (ANRS) 12249 Treatment as Prevention (TasP) trial (ClinicalTrials.gov identifier: NCT01509508), an ongoing cluster-randomized trial evaluating the impact of immediate vs deferred ART initiation (according to South African guidelines) on HIV incidence [20] (see Supplementary Materials). The trial started recruiting in March 2012 in Hlabisa subdistrict, Umkhanyakude district, Northern KwaZulu-Natal, where the antenatal prevalence of HIV is 44%, one of the highest in South Africa [21, 22]. This rural setting is also one of the poorest districts in South Africa with very high unemployment rates.

This analysis is based on the treatment outcomes documented prospectively in the subgroup of patients on second-line bPI-based treatment within the TasP trial. Participants from all clusters irrespective of trial arm were included in this cohort. Participants were aged >15 years, resident in Hlabisa subdistrict, and included from the date of initiation of second-line treatment till the earliest of the date last seen in clinic, death, or loss to follow-up. No participants initiating bPI ART after May 2015 were included to allow at least 6 months' follow-up for the primary outcome to occur. The participants' outcomes were recorded until November 2015.

### Outcome and Prespecified Explanatory Variables

The primary outcome was VF defined as a VL >1000 copies/mL on at least 1 occasion ≥6 months after commencing second-line treatment. Any death occurring during the trial period was recorded and follow-up time was censored at the date of death.

Loss to follow-up was defined as ≥3 consecutive missed clinic appointments. Follow-up time was censored at the last clinic visit. For all the other participants who remained in the trial with virological suppression (VL < 1000 copies/mL), follow-up was censored as the latest of either the date of the last laboratory test or last clinic visit.

### Clinic Procedures and Laboratory Methods

At presentation to the trial clinics, all consenting HIV-infected participants were asked to complete the study questionnaires and underwent clinical examination by trial nurses. CD4 count was measured in TasP clinics using a commercial point-of-care CD4 test (Alere PIMA device tool, Alere Inc, Waltham, Massachusetts). VL was measured using The Abbott m2000 RealTime System with HIV type 1 (HIV-1) VL determination from human plasma of HIV-1-infected individuals in the range of 40–10 000 000 copies/mL (Abbott Molecular Inc, Des Plaines, Illinois). The viral load assay was CE (Conformité Européenne) marked and performed at the Africa Centre laboratory; the laboratory participated in Quality Control for Molecular Diagnostics (QCMD) for VL quality assurance testing. Both CD4 and viral load were measured at baseline, and at months 3 and 6 after ART initiation and every 6 months thereafter. Full-genome deep sequencing was performed after 2 consecutive VL measurements >1000 copies/mL at least 6 months apart following second-line VF, as per adapted protocol from Gall et al [23]. In brief, 4 overlapping fragments, spanning approximately 9 kb of the HIV genome, were amplified and purified as per adapted protocol from Gall et al [23]. The library preparation was done on equimolar pooled amplicons, using the Nextera XT Library preparation kit, followed by sequencing on the Illumina MiSeq platform. Next-generation sequencing (NGS) data were analyzed on Geneious software and a threshold of 2% was used for minority variants detection, with a minimum coverage of 1000 reads. In the case of a single VL >1000 copies/mL, Sanger sequencing was done following Manasa et al's protocol [24]. The external quality assurance for Sanger sequencing was with QCMD. Drug resistance mutations were identified according to the Stanford HIV Drug Resistance Database (<http://hivdb.stanford.edu/>) [25]. We reported mutations where they were detected above 2% frequency. Safety monitoring blood samples were also taken as per protocol [20].

### Statistical Analysis

Participant characteristics were reported using frequency and percentage for categorical variables and median and interquartile range (IQR) for continuous variables. The incidence of second-line failure per 100 person-years was estimated with its 95% confidence interval (CI).

We estimated the cumulative incidence function of VF on second-line treatment, taking into account death and loss to

follow-up as competing risks. Competing risk regression was used to estimate the subdistribution hazard ratio (SHR) of the associations between VF and participant clinical and demographic factors, accounting for the competing risks of death and loss to follow-up, according to the model of Fine and Gray [26]. In the final multivariable model, mutually adjusted estimates of the SHRs were determined by including those factors with evidence of an association in the univariable analysis and a *P* value of <.1. Although age and sex were not significantly associated with VF in the univariable analysis, they were kept in the final model as they were a priori specified confounders. Analysis was done using Stata software version 13.

### Ethical Committee Approval

Ethics approval was granted by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BFC 104/11) and the Medicines Control Council of South Africa. The study was also authorized by the KwaZulu-Natal Department of Health in South Africa. Written informed consent was obtained from all participants.

## RESULTS

One hundred one participants were included in this analysis. Sixteen (15.8%) individuals were already on second-line treatment at enrollment into the trial for a median of 2.7 years (IQR, 1.1–3.9 years). Three of these participants had VF at the baseline clinic visit. Seven (6.9%) participants had not initiated ART at enrollment into the trial. The remaining 78 (77.2%) were on first-line NNRTI-based ART for a median duration of 4.9 years (IQR, 3.2–6.7 years) at the time of enrollment; 41 (52.6%) of these had VF at the baseline clinic visit in the TasP trial, necessitating a switch to bPI along with 2 nucleoside reverse transcriptase inhibitors (NRTIs). Median duration on bPI for the 85 patients was 0.6 years (IQR, 0.3–0.9 years).

The majority of participants were female (65.4%) and the median age at initiation of second-line treatment was 37.4 years (IQR, 31.6–45.3 years). There was a high level of unemployment (91.9%) in this cohort of participants residing in a rural setting (Table 1). Thirteen individuals (12.9%) were diagnosed with TB during the study period.

### Virological Failure and Associated Risk Factors

The 101 participants contributed 178.7 person-years of follow-up to the analysis. The overall incidence of VF on second-line ART was 12.9 per 100 person-years. At administrative censoring, 76 participants were alive and in care, 1 had died, and 1 was lost to follow-up before any VF was documented, and 23 participants had VF at least 6 months after initiating bPI second-line ART (Supplementary Figure 1). Following intensification of adherence counseling, 13 of the 23 participants (56.5%), including the 3 patients with bPI VF at the baseline clinic visit,

**Table 1. Demographic and Clinical Characteristics of Study Participants**

| Characteristic   | No. (%)           |
|--|-------------------|
| Sex (n = 101)  |                   |
| Female   | 66 (65.4)         |
| Age at initiating bPI-based ART, y, median (IQR) (n = 101)                             | 37.4 (31.6–45.3)  |
| Relationship status (n = 100)  |                   |
| Single   | 76 (76.0)         |
| Married  | 17 (17.0)         |
| Widowed  | 7 (7.0)           |
| Employed (n = 87)  |                   |
| Yes  | 7 (8.1)           |
| Education level (n = 101)  |                   |
| Primary or less  | 46 (45.5)         |
| Some secondary   | 30 (29.7)         |
| Completed secondary  | 25 (24.8)         |
| Household asset ownership index score (n = 100)  |                   |
| Low  | 37 (37.0)         |
| Medium   | 52 (52.0)         |
| High   | 11 (11.0)         |
| Other HIV-positive household member (n = 101)  |                   |
| Yes  | 68 (67.3)         |
| Distance to national highway, km, median (IQR) (n = 101)                               | 2.5 (1.4–5.6)     |
| Distance to clinic, km, median (IQR) (n = 101)   | 1.2 (0.8–1.9)     |
| Clinical characteristics   |                   |
| Duration of HIV diagnosis, y, median (IQR) (n = 95)                                    | 5.1 (2.7–7.6)     |
| Duration on NNRTI-based first-line ART, y, median (IQR) (n = 101)                      | 4.6 (2.2–6.4)     |
| Duration on bPI-based second-line ART, y, median (IQR) (n = 101)                       | 2.0 (1.4–2.5)     |
| On bPI before recruitment to TasP (n = 101)  |                   |
| Yes  | 16 (15.8)         |
| CD4 within 6 mo prior to switch to bPI, cells/mm <sup>3</sup> , median (IQR) (n = 31)  | 180 (107–343)     |
| Viral load within 6 mo of switch to bPI, copies/mL (n = 61)                            |                   |
| <1000  | 18 (29.5)         |
| >1000  | 43 (70.5)         |
| Nadir CD4 count prior to first-line ART, cells/mm <sup>3</sup> , median (IQR) (n = 94) | 95.5 (17.0–191.0) |
| Tuberculosis treatment within 6 mo of PI failure (n = 94)                              | 5 (5.3)           |
| No. of clinic visits/y, median (IQR) (n = 101)   | 12.7 (10.4–14.0)  |
| WHO stage (n = 90) at cohort baseline  |                   |
| 1  | 47 (53.2)         |
| 2  | 18 (20.0)         |
| 3  | 23 (25.6)         |
| 4  | 2 (2.2)           |
| Median pill count, % (n = 92)  |                   |
| 0–96   | 25 (27.2)         |
| ≥97  | 67 (72.8)         |
| First-line regimen   |                   |
| ZDV/d4T + 3TC/FTC + NVP/EFV  | 54 (53.5)         |
| TDF + 3TC/FTC + NVP/EFV  | 47 (46.5)         |
| Second-line regimen  |                   |
| ZDV/TDF + 3TC/FTC + LPV/r  | 101 (100.0)       |

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy; bPI, ritonavir-boosted protease inhibitor; d4T, stavudine; EFV, efavirenz; FTC, emtricitabine; HIV, human immunodeficiency virus; IQR, interquartile range; LPV/r, lopinavir/ritonavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NVP, nevirapine; TasP, treatment as prevention; TDF, tenofovir disoproxil fumarate; WHO, World Health Organization; ZDV, zidovudine.



resuppressed with a VL <1000 copies/mL after a median of 8.0 months (IQR, 2.8–16.8 months) (Supplementary Figure 2). Eight of these 13 participants subsequently rebounded with a VL >1000 copies/mL. The prevalence of VF in patients alive and on bPI ART at the time of administrative censoring was 17.8% (18/101).

In the univariable analysis, second-line VF was associated with concomitant TB treatment within 6 months of failure (SHR, 15.9 [95% CI, 6.2–40.6];  $P < .0001$ ) and a lower level of adherence (median pill count <97%) (SHR, 2.4 [95% CI, 1.0–5.7];  $P = .04$ ). In the multivariable analysis, the association of TB treatment with VF on bPI second-line treatment remained (SHR, 11.5 [95% CI, 3.9–33.7];  $P < .001$ ), whereas the association with median pill count was no longer present (Table 2).

Thirteen participants were diagnosed with TB and received antituberculosis treatment during the study observation period; 4 of them were treated within 6 months of VF; 1 participant, within 6 months of censoring, did not experience VF; the remaining 8 participants were diagnosed and treated for TB at time points distant from their study exit and did not exhibit VF after initiation of antituberculosis treatment.

#### Drug Resistance

Genotypes were available at first-line NNRTI ART failure in 9 participants and at bPI ART failure in 6 participants of the 23 with VF failure of second-line ART (Table 3). The reasons for missing genotypes were as follows: 4 patients receiving care in Department of Health clinics and therefore no sample was available; resuppression in 10 patients and therefore no viremic confirmatory sample available for genotyping; and no confirmatory sample in 2. Three of 5 patients exposed to tenofovir in their first-line regimen developed high-level tenofovir resistance with the K65R mutation, and 2 had accessory tenofovir mutations A62V, V75I, or F77L in the reverse transcriptase gene. In one of these individuals, K65R was detected only by NGS (at 12% frequency; Table 3). All first-line failures had major NNRTI resistance and 5 of 9 had high-level lamivudine/emtricitabine resistance (M184V/I). An additional drug resistance-associated variant (at <20% frequency) conferring NNRTI resistance was detected in patient 6 (K103N at 12%). NGS did not detect minority variants in the protease gene in any of the 9 patients with second-line failure.

At second-line bPI ART failure, only 1 of 8 (12.5%) participants had acquired major PI mutations: M46I, I54V, L76V, and V82A. This individual received rifampicin containing TB treatment started at the same time as double-dose bPI.

#### DISCUSSION

We determined the incidence rate for VF on second-line ART and associated risk factors in rural KwaZulu-Natal within the TasP trial. The incidence rate of VF was 12.9 per 100 person-years (95% CI, 8.6–19.4), and prevalence was 17.8% at

the end of the observation period. A meta-analysis of studies conducted in resource-limited settings reported a pooled prevalence of VF of 23.1% (range, 11.4%–39.9%) after 12 months of treatment with bPI ART [6], although our prevalence estimate was based on a follow-up period of <1 year in those initiated on second-line ART within TasP. More recent randomized trials reported a lower prevalence of VF, between 14% and 19% at 48 and 96 weeks of treatment [13–15, 27]. However, the lower prevalence within trials may not be generalizable to the “real world” because trial participants are closely monitored.

There are considerable differences between studies in the definition of VF (from VL >50 to >1000 copies/mL), and guidelines are not clear on a definition of second-line failure that should trigger a switch of treatment regimen. We therefore chose a pragmatic definition of a single VL >1000 copies/mL, particularly as the follow-up time was relatively short for patients in TasP who switched to second-line ART within the trial. We found that many patients with VF on bPI ART resuppressed to VL <1000 copies/mL following a period of intense adherence counseling. This phenomenon has been reported in patients treated with first-line ART in both South Africa [28] and other parts of sub-Saharan Africa [29], and demonstrates that the efficacy of second-line treatment could be optimized if regular VL monitoring is widely available, adding to the impetus for development of point-of-care VL testing in tandem with effective adherence counseling.

The association between TB treatment and VF of NNRTI-based first-line ART has previously been reported [17]. Here we found evidence of an association between TB treatment and VF of second-line treatment. Our study design did not allow us to infer causality between TB treatment and VF, as some patients were never virologically suppressed even before the rifampicin treatment. TB disease itself could be a marker of virological and clinical failure; indeed, the 2010 World Health Organization (WHO) clinical criteria for treatment failure include a new diagnosis of TB [30]. In patients failing during the rifampicin treatment, the mechanism leading to VF may be due to drug interactions between PIs and rifampicin. Rifampicin, a potent cytochrome P450 3A4 inducer, significantly reduces the serum levels of PIs, and concurrent use can lead to VF [31, 32]. To compensate for this phenomenon, double-dosed bPI has been proposed—from twice-daily 400 mg/100 mg to 800 mg/200 mg of lopinavir/ritonavir, as is in this clinical setting [33]. However, the success of such a strategy may be limited by intolerance to the higher doses of PIs [34–36]. Nonadherence to treatment may also contribute to failure as the inherent polypharmacy required to treat both conditions may be a challenge for patients.

Poor adherence has been shown to be associated with VF in other studies [6, 8, 10, 17, 18]; however, we found no association between adherence measured by pill count and VF. This might in part be due to the fact that adherence was high in this trial context, although announced pill count is susceptible to pill dumping by

**Table 2. Subdistribution Hazard Ratios (SHRs) of Clinical and Demographic Characteristics and Association With Virological Failure on Second-line Ritonavir-Boosted Protease Inhibitor–Based Treatment: Univariable Analysis Followed by Multivariable Model of Mutually Adjusted SHRs**

| Characteristic   | Univariable Model                  |                            |                    |                     | Multivariable Model |                    |
|--|------------------------------------|----------------------------|--------------------|---------------------|---------------------|--------------------|
|  | Events/Follow-up Time <sup>a</sup> | Rate (95% CI) <sup>b</sup> | SHR (95% CI)       | PValue              | SHR (95% CI)        | PValue             |
| <b>Sex</b>   |                                    |                            |                    |                     |                     |                    |
| Female   | 15/1.29                            | 11.59 (6.99–19.22)         | 1                  | .90                 | 1                   | .79                |
| Male   | 8/0.51                             | 15.72 (7.87–31.45)         | 1.06 (.45–2.49)    |                     | 0.85 (.18–1.64)     |                    |
| <b>Age at initiating bPI-based ART, y</b>                |                                    |                            |                    |                     |                     |                    |
| 16–35  | 11/0.78                            | 14.18 (7.85–25.61)         | 1                  | .61                 | 1                   | .98                |
| ≥35  | 12/1.03                            | 11.68 (6.63–20.56)         | 0.81 (.36–1.82)    |                     | 1.02 (.33–3.04)     |                    |
| <b>Relationship status</b>                               |                                    |                            |                    |                     |                     |                    |
| Single   | 18/1.34                            | 13.48 (8.49–21.39)         | 1                  | .55                 |                     |                    |
| Married  | 3/0.34                             | 8.68 (2.80–26.90)          | 0.72 (.19–2.67)    |                     |                     |                    |
| Widowed  | 2/0.10                             | 20.20 (5.05–80.75)         | 1.72 (.53–5.59)    |                     |                     |                    |
| <b>Education level</b>                                   |                                    |                            |                    |                     |                     |                    |
| Primary or less  | 11/0.84                            | 13.13 (7.27–23.71)         | 1                  | .93                 |                     |                    |
| Some secondary   | 6/0.43                             | 13.85 (6.22–30.83)         | 1.16 (.47–2.84)    |                     |                     |                    |
| Completed secondary                                      | 6/0.53                             | 11.28 (5.07–25.10)         | 1.18 (.41–3.38)    |                     |                     |                    |
| <b>Employed</b>  |                                    |                            |                    |                     |                     |                    |
| No   | 16/1.29                            | 12.41 (7.60–20.26)         | 1                  | .87                 |                     |                    |
| Yes  | 1/0.09                             | 10.65 (1.50–75.62)         | 0.86 (.16–4.79)    |                     |                     |                    |
| <b>Household asset ownership index score</b>             |                                    |                            |                    |                     |                     |                    |
| Low  | 7/0.69                             | 10.20 (4.86–21.39)         | 1                  | .87                 |                     |                    |
| Medium   | 14/0.92                            | 15.18 (8.99–25.63)         | 1.14 (.44–2.93)    |                     |                     |                    |
| High   | 2/0.17                             | 11.55 (2.89–46.20)         | 1.42 (.37–5.47)    |                     |                     |                    |
| <b>Other HIV-positive household member</b>               |                                    |                            |                    |                     |                     |                    |
| No   | 8/0.56                             | 14.29 (7.14–28.57)         | 1                  | .70                 |                     |                    |
| Yes  | 15/1.24                            | 12.07 (7.27–20.12)         | 1.17 (.52–2.61)    |                     |                     |                    |
| <b>Distance to national highway, km</b>                  |                                    |                            |                    |                     |                     |                    |
| <2   | 14/0.69                            | 20.35 (12.05–34.36)        | 1                  | .16                 |                     |                    |
| 2–16   | 9/1.12                             | 8.07 (4.20–15.51)          | 0.54 (.23–1.27)    |                     |                     |                    |
| <b>Distance to clinic, km</b>                            |                                    |                            |                    |                     |                     |                    |
| <1   | 10/0.76                            | 13.23 (7.12–24.58)         | 1                  |                     |                     |                    |
| 1–2  | 8/0.61                             | 13.11 (6.55–26.20)         | 1.78 (.70–4.52)    |                     |                     |                    |
| 2–4  | 5/0.44                             | 11.45 (4.77–27.52)         | 0.81 (.27–2.41)    | .34                 |                     |                    |
| <b>Clinical characteristics</b>                          |                                    |                            |                    |                     |                     |                    |
| Nadir CD4 prior to first-line ART, cells/mm <sup>3</sup> |                                    |                            |                    |                     |                     |                    |
| <100   | 8/0.48                             | 16.78 (8.39–33.56)         | 1                  | .37                 |                     |                    |
| ≥100   | 10/0.93                            | 10.72 (5.77–19.92)         | 0.66 (.26–1.66)    |                     |                     |                    |
| <b>Tuberculosis treatment within 6 mo of PI failure</b>  |                                    |                            |                    |                     |                     |                    |
| No   | 17/1.62                            | 10.47 (6.51–16.84)         | 1                  | <.0001 <sup>c</sup> | 1                   | <.001 <sup>c</sup> |
| Yes  | 4/0.03                             | 116.23 (43.62–309.68)      | 15.86 (6.21–40.56) |                     | 11.50 (3.92–33.74)  |                    |
| <b>No. of visits per year</b>                            |                                    |                            |                    |                     |                     |                    |
| 0–11   | 17/0.91                            | 18.76 (11.67–30.18)        | 1                  | .75                 |                     |                    |
| 12–22  | 6/0.90                             | 6.69 (3.01–14.89)          | 0.85 (.30–2.34)    |                     |                     |                    |
| <b>WHO stage</b>   |                                    |                            |                    |                     |                     |                    |
| 1  | 7/0.80                             | 8.79 (4.19–18.44)          | 1                  | .26                 |                     |                    |
| 2  | 7/0.26                             | 27.12 (12.93–56.89)        | 2.03 (.86–4.81)    |                     |                     |                    |
| 3/4  | 7/0.50                             | 13.94 (6.64–29.24)         | 1.37 (.42–4.51)    |                     |                     |                    |
| <b>Median pill count, %</b>                              |                                    |                            |                    |                     |                     |                    |
| ≥97  | 12/1.30                            | 27.14 (14.12–52.16)        | 1                  | .04 <sup>c</sup>    | 1                   | .28                |
| 0–96   | 9/0.33                             | 9.20 (5.23–16.20)          | 2.41 (1.02–5.65)   |                     | 1.83 (0.61–5.50)    |                    |
| <b>Duration between HIV diagnosis and baseline, y</b>    |                                    |                            |                    |                     |                     |                    |
| 0–3  | 8/0.66                             | 12.21 (6.11–24.42)         | 1                  | .87                 |                     |                    |
| 4–7  | 8/0.64                             | 12.41 (6.21–24.82)         | 0.80 (.31–2.05)    |                     |                     |                    |
| 8–20   | 5/0.36                             | 13.75 (5.73–33.05)         | 1.01 (.32–3.17)    |                     |                     |                    |
| <b>On PI before recruitment to TasP</b>                  |                                    |                            |                    |                     |                     |                    |
| No   | 16/1.31                            | 12.22 (7.49–19.95)         | 1                  | .13                 |                     |                    |
| Yes  | 7/0.49                             | 14.17 (6.76–29.73)         | 1.97 (.81–4.75)    |                     |                     |                    |

Table 2. Continued

| Characteristic                     | Univariable Model                  |                            |                 |         | Multivariable Model |         |
|------------------------------------|------------------------------------|----------------------------|-----------------|---------|---------------------|---------|
|                                    | Events/Follow-up Time <sup>a</sup> | Rate (95% CI) <sup>b</sup> | SHR (95% CI)    | P Value | SHR (95% CI)        | P Value |
| Duration on first-line regimen, y  |                                    |                            |                 |         |                     |         |
| <3                                 | 7/0.61                             | 11.50 (5.48–24.12)         | 1               | .35     |                     |         |
| 3–5                                | 7/0.73                             | 9.58 (4.67–20.09)          | 0.69 (.24–1.94) |         |                     |         |
| 6–12                               | 9/0.46                             | 19.42 (10.11–37.33)        | 1.38 (.50–3.84) |         |                     |         |
| Duration on second-line regimen, y |                                    |                            |                 |         |                     |         |
| <2                                 | 8/0.56                             | 14.18 (7.09–28.36)         | 1               | .72     |                     |         |
| 2–3                                | 9/0.70                             | 12.87 (6.70–24.73)         | 0.99 (.38–2.56) |         |                     |         |
| 3–10                               | 6/0.54                             | 11.12 (5.00–24.75)         | 1.47 (.49–4.40) |         |                     |         |

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ART, antiretroviral therapy; bPI, ritonavir-boosted protease inhibitor; CI, confidence interval; HIV, human immunodeficiency virus; PI, protease inhibitor; SHR, subdistribution hazard ratio; TasP, treatment as prevention; WHO, World Health Organization.

<sup>a</sup>Follow-up time in 100 person-years.

<sup>b</sup>Rate per 100 person-years.

<sup>c</sup>Associations with some evidence against the null.

participants and therefore not be a particularly good marker for adherence: indeed, 88% of participants on first-line NNRTI ART had an overall adherence of  $\geq 95\%$  at 6 months [37].

We found multiple major NRTI and NNRTI mutations at first-line failure in the participants who had genotype testing (9 participants), consistent with data from resource-limited

settings [2, 38]. Though numbers were small, NGS increased detection of significant tenofovir resistance (K65R mutation) by around 50%, consistent with previous NGS studies in this setting [39]. We found acquired major protease mutations in only 1 (11%) failing bPI ART, consistent with other data from South Africa [8, 18, 40–42]. NGS did not increase the detection rate of

Table 3. Resistance Mutations Identified by Next-Generation Sequencing

| Participant ID | First-line Regimen             | Second-line Regimen | Duration on bPI, y | Time-point           | PI Mutations                  | NRTI Mutations  | NNRTI Mutations  |
|----------------|--------------------------------|---------------------|--------------------|----------------------|-------------------------------|---|--|
| 1              | d4T, 3TC, EFV                  | TDF, 3TC, LPV/r     | 2.0                | First-line failure   | —                             | M184V   | K103N, P225H, K238T                                      |
|                |                                |                     |                    | Second-line failure  | —                             | —   | <i>P225H</i> <sub>10%</sub> , <i>K238T</i> <sub>8%</sub> |
| 2              | TDF, FTC, EFV                  | ZDV, 3TC, LPV/r     | 2.3                | First-line failure   | —                             | —   | —  |
|                |                                |                     |                    | Second-line failure  | —                             | —   | —  |
| 3              | TDF, 3TC, EFV                  | TDF, 3TC, LPV/r     | 1.1                | First-line failure   | —                             | A62V, K65R, V75I, Y115F                                   | E138Q, G190E   |
|                |                                |                     |                    | Second-line failure* | —                             | —   | —  |
| 4              | d4T, 3TC, NVP<br>d4T, 3TC, EFV | TDF, 3TC, LPV/r     | 1.7                | First-line failure   | —                             | M184V   | K103N, P255H   |
|                |                                |                     |                    | Second-line failure  | —                             | —   | —  |
| 5              | d4T, 3TC, EFV                  | TDF, 3TC, LPV/r     | 1.9                | First-line failure   | —                             | T69N, K70N  | V106M, <i>E138G</i> <sub>6%</sub> , G190A, F227L         |
|                |                                |                     |                    | Second-line failure  | —                             | —   | —  |
| 6              | TDF, 3TC, EFV                  | ZDV, 3TC, LPV/r     | 2.1                | First-line failure   | —                             | —   | <i>K103N</i> <sub>12%</sub> , V106M, G190A               |
|                |                                |                     |                    | Second-line failure  | —                             | —   | —  |
| 7              | d4T, 3TC, EFV<br>ZDV, 3TC, EFV | TDF, 3TC, LPV/r     | 1.6                | First-line failure   | —                             | M41L, L74I, V75L, M184V, T215Y                            | V106M, V179D   |
|                |                                |                     |                    | Second-line failure  | —                             | —   | —  |
| 8              | d4T, 3TC, EFV<br>TDF, 3TC, EFV | ZDV, 3TC, LPV/r     | 2.0                | First-line failure   | —                             | M41L, A62V, K65R, <i>K70T</i> <sub>2%</sub> , V75I, M184V | K103N, V106M, <i>E138G</i> , <i>F227L</i> <sub>6%</sub>  |
|                |                                |                     |                    | Second-line failure  | <b>M46I, I54V, L76V, V82A</b> | <b>T215Y</b>  | <i>E138G</i> <sub>5%</sub>                               |
| 9              | d4T, 3TC, NVP<br>TDF, FTC, EFV | TDF, 3TC, LPV/r     | 1.5                | First-line failure   | —                             | <i>K65R</i> <sub>12%</sub> , M184V                        | K103N, Y188L/ <i>F</i> <sub>15%</sub> , K238T            |
|                |                                |                     |                    | Second-line failure  | —                             | —   | —  |

First-line failure time-point indicates mutations present at first-line NNRTI virological failure. Second-line failure time-point indicates mutations acquired or lost where there are second-line bPI failure sequencing data available. These are presented in boldface type where they are newly acquired and where a mutation is lost. Minority variants detected between 2% and 20% are reported in italic type with their respective frequencies in subscript. Sequences with an asterisk (\*) indicate population sequencing data derived by Sanger methodology. Dash (—) indicates no mutations.

Abbreviations: 3TC, lamivudine; bPI, ritonavir-boosted protease inhibitor; d4T, stavudine; EFV, efavirenz; FTC, emtricitabine; ID, Identity Document; LPV/r, lopinavir/ritonavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.

mutations in protease. The high genetic barrier of bPI to resistance development could be a reason for this. It is worth noting that standard genotype tests based on *pol* sequencing ignore the influence of mutations in other genes such as *gag* [43–47] and *env* [48] on PI resistance. Notably, the individual with major protease resistance had received double-boosted PI treatment, and coupled with previous reports of multiple major protease resistance mutations in children treated with double-dose PI [49, 50], further work regarding this approach is warranted.

The main methodological strength of this study is the application of regression methods, which account for the presence of competing risks to estimate the rate of VF on second-line treatment and the association between covariates of interest and VF. Other cohort studies reporting outcomes on second-line treatment and factors associated with VF have used standard Kaplan-Meier survival analysis and Cox regression models, which can lead to biased or inflated estimates of association. As this analysis was done on prospectively collected data within a trial context with preset procedures rather than routine data, we also minimized the common problem of missing data and information bias.

There are some limitations of the study. First, although the sample size is small, the prevalence of VF on bPI we found is in line with other published studies. The inclusion of whole-genome sequencing data, albeit from 9 patients, is unique to this cohort. Second, the study was nested in a randomized community trial and as such the patients may not be truly reflective of the general HIV population, given the intense monitoring and provision of counseling and adherence interventions that may impact their attitude toward health and promote more successful treatment outcomes. We did not measure drug levels to assess pharmacokinetic drug–drug interactions or adherence before a clinical visit. Finally, 16 (15.8%) patients were included who were already on second-line ART at enrollment, for a median of 2.7 years, representing a potential bias.

There was also heterogeneity in the patient population. We included all participants from the parent study irrespective of study arm, though study arm had no effect on outcome, largely due to poor linkage to care in this setting (Dabis et al, 21st International AIDS Conference 2016, Durban, abstract FRAC0105LB). We included newly diagnosed as well as ART-experienced patients, as both present for first-line therapy under “real-world” programmatic conditions, sometimes with evidence of drug resistance to thymidine analogues arising from prior ART [51] or with evidence of transmitted drug resistance [52].

In conclusion, this study found that second-line PI-based VF was common in this population accessing ART in rural South Africa under trial circumstances but recruited at the population level. Further research is needed to understand the mechanisms behind VF of bPI ART in TB-coinfected patients. Novel approaches to optimize second-line ART in resource-limited

settings are still urgently needed as this population is likely to grow rapidly, owing to the WHO 2015 guidelines to test and treat all people living with HIV [53].

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

**Author contributions.** D. C., R. K. G., F. D., D. P., and C. I. designed the study; R. G., D. C., N. O., C. I., F. D., A. C., and D. P. collected and analyzed the data; D. C. and R. G. wrote the first draft; A. D. and T. de O. performed experiments and analyzed data.

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## References

- Boender TS, Sigaloff KC, McMahon JH, et al. Long-term virological outcomes of first-line antiretroviral therapy for HIV-1 in low- and middle-income countries: a systematic review and meta-analysis. *Clin Infect Dis* 2015; 61:1453–61.
- Gupta RK, Hill A, Sawyer AW, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. *Lancet Infect Dis* 2009; 9:409–17.
- Hamers RL, Sigaloff KC, Wensing AM, et al; PharmAccess African Studies to Evaluate Resistance (PASER). Patterns of HIV-1 drug resistance after first-line antiretroviral therapy (ART) failure in 6 sub-Saharan African countries: implications for second-line ART strategies. *Clin Infect Dis* 2012; 54:1660–9.
- Manasa J, Lessells RJ, Skingsley A, et al; Southern African Treatment and Resistance Network. High-levels of acquired drug resistance in adult patients failing first-line antiretroviral therapy in a rural HIV treatment programme in KwaZulu-Natal, South Africa. *PLoS One* 2013; 8:e72152.
- Estill J, Ford N, Salazar-Vizcaya L, et al. The need for second-line antiretroviral therapy in adults in sub-Saharan Africa up to 2030: a mathematical modelling study. *Lancet HIV* 2016; 3: e132–9.
- Ajose O, Mookerjee S, Mills EJ, Boule A, Ford N. Treatment outcomes of patients on second-line antiretroviral therapy in resource-limited settings: a systematic review and meta-analysis. *AIDS* 2012; 26:929–38.
- Fox MP, Iye P, Long L, Maskew M, Sanne I. High rates of survival, immune reconstitution, and virologic suppression on second-line antiretroviral therapy in South Africa. *J Acquir Immune Defic Syndr* 2010; 53:500–6.
- Johnston V, Cohen K, Wiesner L, et al. Viral suppression following switch to second-line antiretroviral therapy: associations with nucleoside reverse transcriptase inhibitor resistance and subtherapeutic drug concentrations prior to switch. *J Infect Dis* 2014; 209:711–20.
- Levison JH, Orrell C, Losina E, Lu Z, Freedberg KA, Wood R. Early outcomes and the virological effect of delayed treatment switching to second-line therapy in an antiretroviral roll-out programme in South Africa. *Antivir Ther* 2011; 16:853–61.



10. Murphy RA, Sunpath H, Castilla C, et al. Second-line antiretroviral therapy: long-term outcomes in South Africa. *J Acquir Immune Defic Syndr* **2012**; 61:158–63.
11. Schoffelen AF, Wensing AM, Tempelman HA, Geelen SP, Hoepelman AI, Barth RE. Sustained virological response on second-line antiretroviral therapy following virological failure in HIV-infected patients in rural South Africa. *PLoS One* **2013**; 8:e58526.
12. Sigaloff KC, Hamers RL, Wallis CL, et al; PharmAccess African Studies to Evaluate Resistance (PASER). Second-line antiretroviral treatment successfully resuppresses drug-resistant HIV-1 after first-line failure: prospective cohort in sub-Saharan Africa. *J Infect Dis* **2012**; 205:1739–44.
13. Paton NI, Kityo C, Hoppe A, et al; EARNEST Trial Team. Assessment of second-line antiretroviral regimens for HIV therapy in Africa. *N Engl J Med* **2014**; 371:234–47.
14. Ciaffi L, Koulla-Shiro S, Sawadogo A, et al; 2LADY Study Group. Efficacy and safety of three second-line antiretroviral regimens in HIV-infected patients in Africa. *AIDS* **2015**; 29:1473–81.
15. Amin J, Boyd MA, Kumarasamy N, et al; SECOND-LINE. Correction: raltegravir non-inferior to nucleoside based regimens in second-line therapy with lopinavir/ritonavir over 96 weeks: a randomised open label study for the treatment of HIV-1 infection. *PLoS One* **2015**; 10:e0140623.
16. Hakim JT, Kityo CM, Walker S, et al. Second-line treatment in sub-Saharan Africa: week 144 follow-up of the earnest trial. *Top Antiviral Med* **2015**; 23:232.
17. El-Khatib Z, Ekstrom AM, Ledwaba J, et al. Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. *AIDS* **2010**; 24:1679–87.
18. Lathouwers E, De Meyer S, Dierynck I, et al. Virological characterization of patients failing darunavir/ritonavir or lopinavir/ritonavir treatment in the ARTEMIS study: 96-week analysis. *Antivir Ther* **2011**; 16:99–108.
19. Gupta A, Nadkarni G, Yang WT, et al. Early mortality in adults initiating antiretroviral therapy (ART) in low- and middle-income countries (LMIC): a systematic review and meta-analysis. *PLoS One* **2011**; 6:e28691.
20. Iwuji CC, Orne-Gliemann J, Tanser F, et al; ANRS 12249 TasP Study Group. Evaluation of the impact of immediate versus WHO recommendations-guided antiretroviral therapy initiation on HIV incidence: the ANRS 12249 TasP (treatment as prevention) trial in Hlabisa sub-district, KwaZulu-Natal, South Africa: study protocol for a cluster randomised controlled trial. *Trials* **2013**; 14:230.
21. National Department of Health. The 2013 National Antenatal Sentinel HIV Prevalence Survey South Africa. Pretoria: National Department of Health, **2015**.
22. Houlihan CF, Bland RM, Mutevedzi PC, et al. Cohort profile: Hlabisa HIV treatment and care programme. *Int J Epidemiol* **2011**; 40:318–26.
23. Gall A, Ferns B, Morris C, et al. Universal amplification, next-generation sequencing, and assembly of HIV-1 genomes. *J Clin Microbiol* **2012**; 50:3838–44.
24. Manasa J, Danaviah S, Pillay S, et al. An affordable HIV-1 drug resistance monitoring method for resource limited settings. *J Vis Exp* **2014**. doi:10.3791/51242.
25. Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic Acids Res* **2003**; 31:298–303.
26. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* **1999**; 94: 496–509.
27. Boyd MA, Kumarasamy N, Moore CL, et al; SECOND-LINE Study Group. Ritonavir-boosted lopinavir plus nucleoside or nucleotide reverse transcriptase inhibitors versus ritonavir-boosted lopinavir plus raltegravir for treatment of HIV-1 infection in adults with virological failure of a standard first-line ART regimen (SECOND-LINE): a randomised, open-label, non-inferiority study. *Lancet* **2013**; 381:2091–9.
28. Hoffmann CJ, Charalambous S, Sim J, et al. Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa. *Clin Infect Dis* **2009**; 49:1928–35.
29. Gupta RK, Goodall RL, Ranopa M, et al; DART Virology Group and Trial Team. High rate of HIV resuppression after viral failure on first-line antiretroviral therapy in the absence of switch to second-line therapy. *Clin Infect Dis* **2014**; 58:1023–6.
30. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach: 2010 revision. Geneva, Switzerland: WHO, **2010**.
31. Burger DM, Agarwala S, Child M, Been-Tiktak A, Wang Y, Bertz R. Effect of rifampin on steady-state pharmacokinetics of atazanavir with ritonavir in healthy volunteers. *Antimicrob Agents Chemother* **2006**; 50:3336–42.
32. la Porte CJ, Colbers EP, Bertz R, et al. Pharmacokinetics of adjusted-dose lopinavir-ritonavir combined with rifampin in healthy volunteers. *Antimicrob Agents Chemother* **2004**; 48:1553–60.
33. National Department of Health. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults. South Africa: National Department of Health **2014**. <http://www.hst.org.za/publications/national-consolidated-guidelines-prevention-mother-child-transmission-hiv-pmtct-and-man>. Accessed 1 August 2016.
34. Murphy RA, Marconi VC, Gandhi RT, Kuritzkes DR, Sunpath H. Coadministration of lopinavir/ritonavir and rifampicin in HIV and tuberculosis co-infected adults in South Africa. *PLoS One* **2012**; 7:e44793.
35. Nijland HM, L'homme RF, Rongen GA, et al. High incidence of adverse events in healthy volunteers receiving rifampicin and adjusted doses of lopinavir/ritonavir tablets. *AIDS* **2008**; 22:931–5.
36. Sunpath H, Winterheimer P, Cohen S, et al. Double-dose lopinavir-ritonavir in combination with rifampicin-based anti-tuberculosis treatment in South Africa. *Int J Tuberc Lung Dis* **2014**; 18:689–93.
37. Iwuji C, Dray-Spira R, Calmy A, et al. Does a universal test and treat strategy impact ART adherence in rural South Africa? ANRS 12249 TasP cluster-randomized trial. *J Int AIDS Soc* **2015**; 18: 16–7.
38. TenoRes Study Group. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. *Lancet Infect Dis* **2016**; 16: 565–75.
39. Casadellà M, Noguera-Julian M, Sunpath H, et al. Treatment options after virological failure of first-line tenofovir-based regimens in South Africa: an analysis by deep sequencing. *AIDS* **2016**; 30:1137–40.
40. Levison JH, Orrell C, Gallien S, et al. Virologic failure of protease inhibitor-based second-line antiretroviral therapy without resistance in a large HIV treatment program in South Africa. *PLoS One* **2012**; 7:e32144.
41. Wallis CL, Mellors JW, Venter WD, Sanne I, Stevens W. Protease inhibitor resistance is uncommon in HIV-1 subtype C infected patients on failing second-line lopinavir/r-containing antiretroviral therapy in South Africa. *AIDS Res Treat* **2011**; 2011:769627.
42. Boender TS, Hamers RL, Ondoa P, et al. Protease inhibitor resistance in the first 3 years of second-line antiretroviral therapy for HIV-1 in sub-Saharan Africa. *J Infect Dis* **2016**; 214:873–83.
43. Fun A, Wensing A, Verheyen J, Nijhuis M. Human immunodeficiency virus Gag and protease: partners in resistance. *Retrovirology* **2012**; 9:63.
44. Gupta RK, Kohli A, McCormick AL, Towers GJ, Pillay D, Parry CM. Full-length HIV-1 Gag determines protease inhibitor susceptibility within in vitro assays. *AIDS* **2010**; 24:1651–5.
45. Parry CM, Kohli A, Boinett CJ, Towers GJ, McCormick AL, Pillay D. Gag determinants of fitness and drug susceptibility in protease inhibitor-resistant human immunodeficiency virus type 1. *J Virol* **2009**; 83:9094–101.
46. Sutherland KA, Mbisa JL, Ghosn J, et al. Phenotypic characterization of virological failure following lopinavir/ritonavir monotherapy using full-length gag-protease genes. *J Antimicrob Chemother* **2014**; 69:3340–8.
47. Sutherland KA, Ghosn J, Gregson J, et al. HIV-1 subtype influences susceptibility and response to monotherapy with the protease inhibitor lopinavir/ritonavir. *J Antimicrob Chemother* **2015**; 70:243–8.
48. Rabi SA, Laird GM, Durand CM, et al. Multi-step inhibition explains HIV-1 protease inhibitor pharmacodynamics and resistance. *J Clin Invest* **2013**; 123:3848–60.
49. Lange CM, Hué S, Violarì A, et al. Single genome analysis for the detection of linked multiclass drug resistance mutations in HIV-1-infected children after failure of protease inhibitor-based first-line therapy. *J Acquir Immune Defic Syndr* **2015**; 69:138–44.
50. Giandhari J, Basson AE, Coovadia A, et al. Genetic changes in HIV-1 Gag-protease associated with protease inhibitor-based therapy failure in pediatric patients. *AIDS Res Hum Retroviruses* **2015**; 31:776–82.
51. Gregson J, Kaleebu P, Marconi VC, et al. Occult drug resistance to thymidine analogues and multidrug resistant HIV-1 following failure of first line tenofovir-based antiretroviral regimens in sub-Saharan Africa: a retrospective multi-centre cohort study. *Lancet Infect Dis* **2016**; doi:10.1016/S1473-3099(16)30469-8.
52. Gupta RK, Jordan MR, Sultan BJ, et al. Global trends in antiretroviral resistance in treatment-naïve individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *Lancet* **2012**; 380:1250–8.
53. World Health Organization. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis to ART. Available at: [http://apps.who.int/iris/bitstream/10665/186275/1/9789241509565\\_eng.pdf?ua=1](http://apps.who.int/iris/bitstream/10665/186275/1/9789241509565_eng.pdf?ua=1). Accessed 1 August 2016.

## APPENDIX

**Appendix Table 1. ANRS 12249 Treatment as Prevention Study Group (as of March 2016)**

| Name                 | Role   | Affiliation   |
|----------------------|--|---|
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| Eric Balestre        | Epidemiology and Biostatistics                     | - Univ. Bordeaux, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France<br>- INSERM, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France                                      |
| Sylvie Boyer         | Health economics                                   | - INSERM, UMR912 (SESSTIM), Marseille, France<br>- Aix Marseille Université, UMR_S912, IRD, Marseille, France<br>- ORS PACA, Observatoire Régional de la Santé Provence-Alpes-Côte d'Azur, Marseille, France        |
| Alexandra Calmy      | Adult Medicine                                     | - Service des maladies infectieuses, Hôpital Universitaire de Genève, Genève, Switzerland   |
| Vincent Calvez       | Virology   | - Department of virology, Hôpital Pitié-Salpêtrière, Paris, France  |
| Anne Derache         | Virology   | - Africa Centre for Population Health, University of KwaZulu-Natal, South Africa  |
| Hermann Donfouet     | Statistics/Economist                               | - INSERM, UMR912 (SESSTIM), Marseille, France<br>- Aix Marseille Université, UMR_S912, IRD, Marseille, France<br>- ORS PACA, Observatoire Régional de la Santé Provence-Alpes-Côte d'Azur, Marseille, France        |
| Rosemary Dray-Spira  | Social sciences                                    | - INSERM U1018, CESP, Epidemiology of Occupational and Social Determinants of Health, Villejuif, France<br>- University of Versailles Saint-Quentin, UMRS 1018, Villejuif, France                                   |
| Jaco Dreyer          | Data management                                    | - Africa Centre for Population Health, University of KwaZulu-Natal, South Africa  |
| Andrea Grosset       | Statistics   | - INSERM, UMR912 (SESSTIM), 13006, Marseille, France<br>- Aix Marseille Université, UMR_S912, IRD, Marseille, France<br>- ORS PACA, Observatoire Régional de la Santé Provence-Alpes-Côte d'Azur, Marseille, France |
| Kobus Herbst         | Data management                                    | - Africa Centre for Population Health, University of KwaZulu-Natal, South Africa  |

**Appendix Table 1. Continued**

| Name                                  | Role                           | Affiliation  |
|---------------------------------------|--------------------------------|--|
| John Imrie                            | Social sciences                | - Africa Centre for Population Health, University of KwaZulu-Natal, South Africa<br>- Centre for Sexual Health and HIV Research, Research Department of Infection and Population, Faculty of Population Health Sciences, University College London, London, UK                             |
| Joseph Larmarange                     | Social sciences                | - CEPED (Centre Population & Développement-UMR 196-Paris Descartes/INED/IRD), IRD (Institut de Recherche pour le Développement), Paris, France.<br>- Africa Centre for Population Health, University of KwaZulu-Natal, South Africa  |
| France Lert                           | Social Sciences                | - INSERM U1018, CESP, Epidemiology of Occupational and Social Determinants of Health, Villejuif, France<br>- University of Versailles Saint-Quentin, UMRS 1018, Villejuif, France  |
| Thembisa Makowa                       | Field operations               | - Africa Centre for Population Health, University of KwaZulu-Natal, South Africa   |
| Anne-Geneviève Marcelin               | Virology                       | - Department of virology, Hôpital Pitié-Salpêtrière, Paris, France   |
| Nuala McGrath                         | Epidemiology/Social sciences   | - Faculty of Medicine and Faculty of Human, Social and Mathematical Sciences, University of Southampton, UK<br>- Africa Centre for Population Health, University of KwaZulu-Natal, South Africa<br>- Research Department of Infection and Population Health, University College London, UK |
| Nonhlanhla Okesola                    | Nurse manager                  | - Africa Centre for Population Health, University of KwaZulu-Natal, South Africa   |
| Tulio de Oliveira                     | Bioinformatics                 | - Africa Centre for Population Health, University of KwaZulu-Natal, South Africa   |
| Delphine Perriat                      | Epidemiology/social sciences   | - Univ. Bordeaux, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France<br>- INSERM, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France   |
| Melanie Plazy                         | Epidemiology/social sciences   | - Univ. Bordeaux, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France<br>- INSERM, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France   |
| Camelia Protopopescu                  | Statistics/Economist           | - INSERM, UMR912 (SESSTIM), Marseille, France<br>- Aix Marseille Université, UMR_S912, IRD, Marseille, France<br>- ORS PACA, Observatoire Régional de la Santé Provence-Alpes-Côte d'Azur, Marseille, France   |
| Luis Sagaon-Teyssier                  | Health economics               | - INSERM, UMR912 (SESSTIM), Marseille, France<br>- Aix Marseille Université, UMR_S912, IRD, Marseille, France<br>- ORS PACA, Observatoire Régional de la Santé Provence-Alpes-Côte d'Azur, Marseille, France   |
| Bruno Spire                           | Health economics               | - INSERM, UMR912 (SESSTIM), 13006, Marseille, France<br>- Aix Marseille Université, UMR_S912, IRD, Marseille, France<br>- ORS PACA, Observatoire Régional de la Santé Provence-Alpes-Côte d'Azur, Marseille, France  |
| Frank Tanser                          | Epidemiology and Biostatistics | - Africa Centre for Population Health, University of KwaZulu-Natal, South Africa   |
| Rodolphe Thiébaud                     | Epidemiology and Biostatistics | - Univ. Bordeaux, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France<br>- INSERM, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France   |
| Thierry Tiendrebeogo                  | Epidemiology and Biostatistics | - Univ. Bordeaux, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France<br>- INSERM, ISPED, Centre Inserm U1219 Bordeaux Population Health, Bordeaux, France   |
| Thembelihle Zuma                      | Psychology/Social sciences     | - Africa Centre for Population Health, University of KwaZulu-Natal, South Africa   |
| Scientific advisory board             |                                |  |
| Chair: Bernard Hirschel (Switzerland) |                                |  |
| International experts                 |                                |  |

**Appendix Table 1. Continued**

| Name                                   | Role | Affiliation |
|--|------|-------------|
| Xavier Anglaret (Ivory Coast)          |      |             |
| Hoosen Coovadia (South Africa)         |      |             |
| Alpha Diallo (France)                  |      |             |
| Bruno Giraudeau (France)               |      |             |
| Jean-Michel Molina (France)            |      |             |
| Lynn Morris (South Africa)             |      |             |
| François Venter (South Africa)         |      |             |
| Sibongile Zungu (South Africa)         |      |             |
| Community representatives              |      |             |
| Eric Fleutelot (France)                |      |             |
| Eric Goemaere (South Africa)           |      |             |
| Calice Talom (Cameroon)                |      |             |
| Sponsor representatives (ANRS)         |      |             |
| Brigitte Bazin                         |      |             |
| Claire Rekacewicz                      |      |             |
| Pharmaceutical company representatives |      |             |
| Golriz Pahlavan-Grumel (MSD)           |      |             |
| Alice Jacob (Gilead)                   |      |             |
| Data safety and monitoring board       |      |             |
| Chair: Patrick Yeni (France)           |      |             |
| Sinead Delany-Moretlwe (South Africa)  |      |             |
| Nathan Ford (South Africa)             |      |             |
| Catherine Hankins (Netherlands)        |      |             |
| Helen Weiss (UK)                       |      |             |